



REPLACEMENT SHEET  
Heller Ehrman White & McAuliffe, LLP  
Sheet 1 of 13  
Title: Positional Sequencing by Hybridization.  
Applicants: Cantor *et al.* Attorney Docket No. 25491-2401G  
U.S. Serial No.: 09/030,571 Filing Date: February 24, 1998

FIG. 1

NUCLEIC ACID  
STRUCTURE

CALCULATED  $T_m$  ( $^{\circ}\text{C}$ , AVERAGE BASE COMPOSITION)

$n = 8 \quad 7 \quad 6 \quad 5$

— — — —	38	33	25	15
— — — —	33	25	15	3
— — — —	25	15	3	-14
— —   — —	51	46	40	31
— —   — —	46	40	31	21
— — — —	40	41	21	11



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FIG. 2A

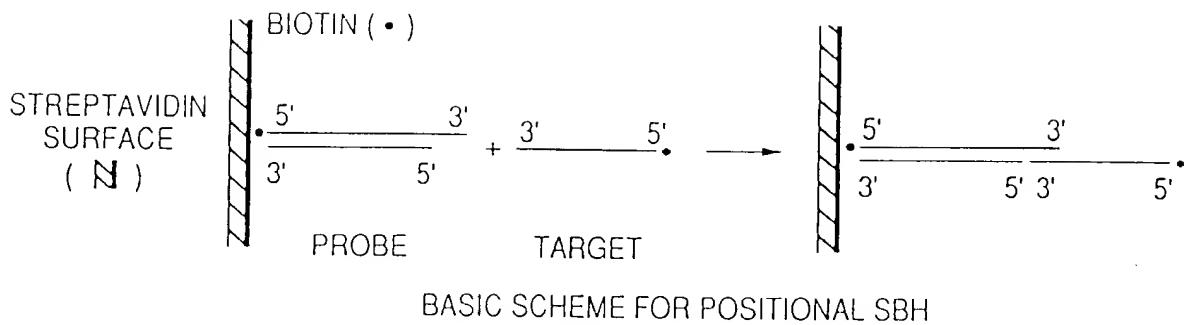
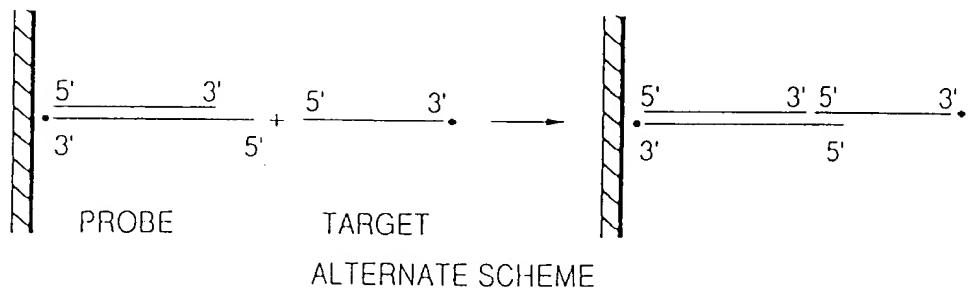


FIG. 2B





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FIG. 3A

LIGATION OF TARGET DNA WITH PROBE

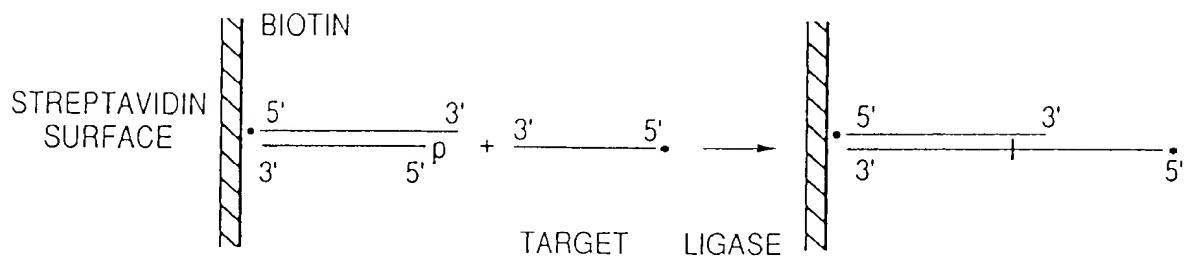
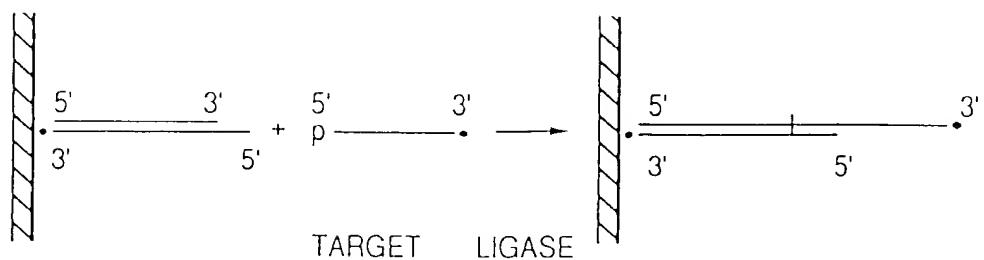


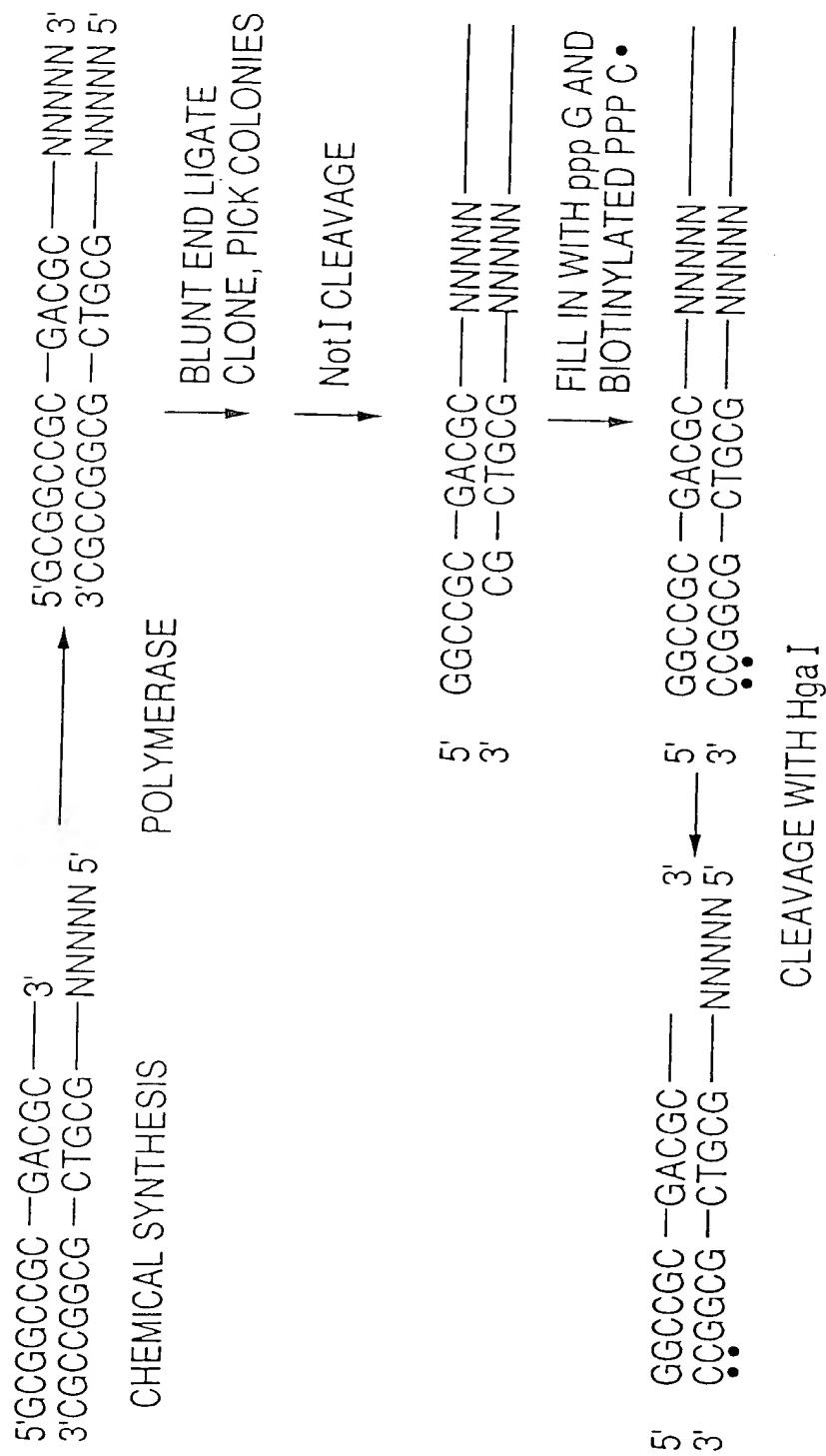
FIG. 3B





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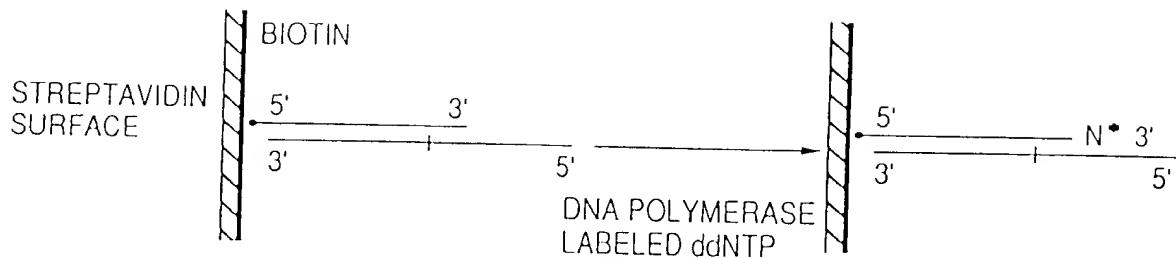
FIG. 4





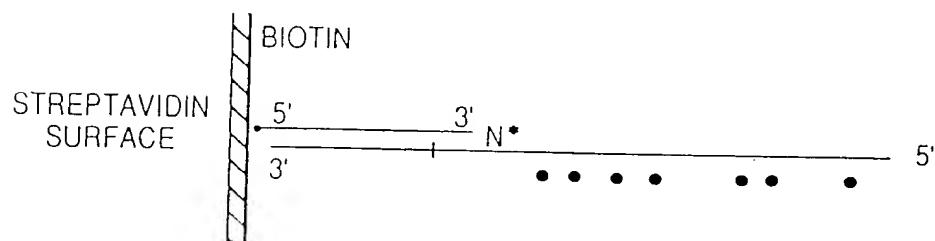
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FIG. 5



READING AN EXTRA TARGET BASE BY 3' EXTENSION OF THE PROBE

FIG. 7



POSITIONAL INFORMATION FROM RATIO OF INTERNAL LABEL (•) TO EXTENSION LABEL (•). A 5' LABEL COULD ALSO BE USED.

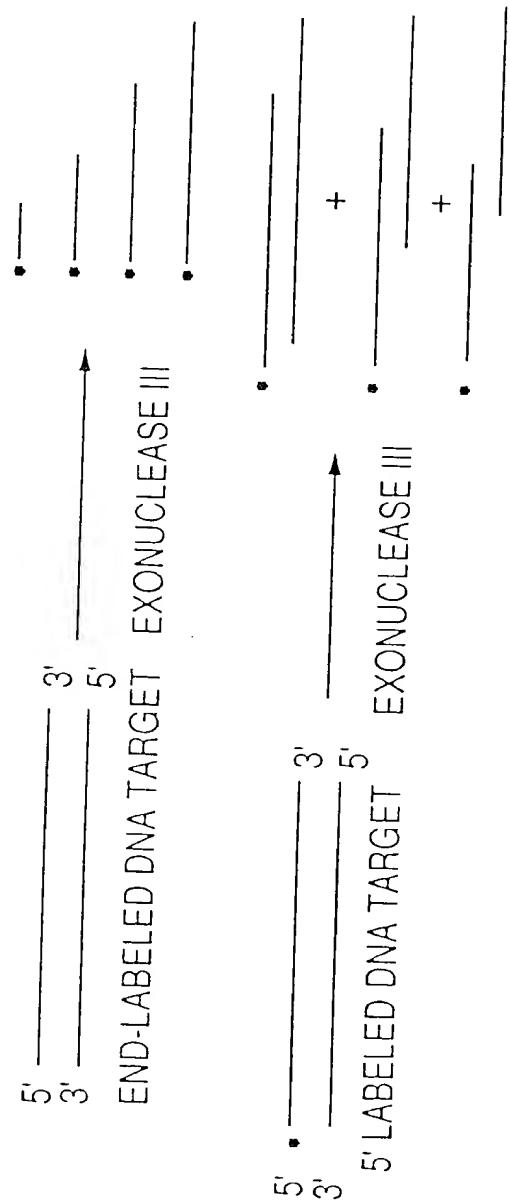


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FIG. 6



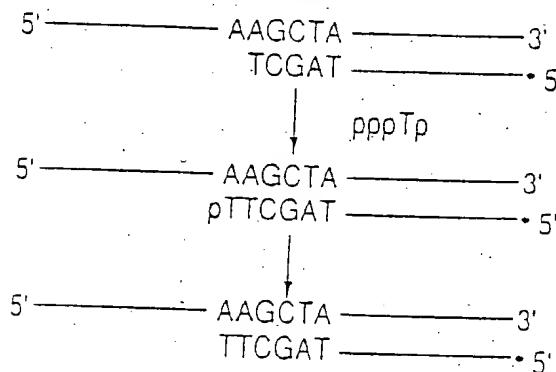
PREPARATION OF A NESTED SET OF DNAs



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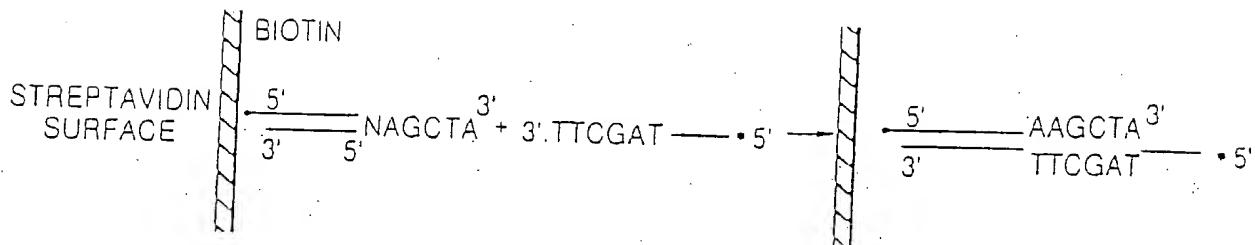
### FIG. 8

#### READING AN EXTRA TARGET BASE



EXTENSION WILL FAIL WITH pppAp, pppGp, AND pppCp

A. 3' EXTENSION OF THE TARGET WITH A pppNp PRIOR TO PHOSPHATASE TREATMENT



ARRAY WITH ONE DEGENERATE BASE

B. HYBRIDIZATION AND LIGATION



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### FIG. 9

#### LABEL INTENSITY

CHAIN TERMINATOR: SEQUENCE	T	G	A
GGAAT	2A, 2G	—	2G
AAGGT	2A, 2G	2A	—
GAAGT	2A, 2G	—	1G
AGGAT	2A, 2G	1A	—
AGAGT	2A, 2G	1A	—
GAGAT	2A, 2G	—	1G
GGGAT	1A, 3G	—	3G
GGAGT	1A, 3G	—	2G
GAGGT	1A, 3G	—	1G
AGGGT	1A, 3G	1A	—
AAAGT	3A, 1G	3A	—
AAGAT	3A, 1G	2A	—
AGAAT	3A, 1G	1A	—
GAAAT	3A, 1G	—	1G
GGGGT	4G	—	1T, 4G*
AAAAT	4A	1T, 4*	—

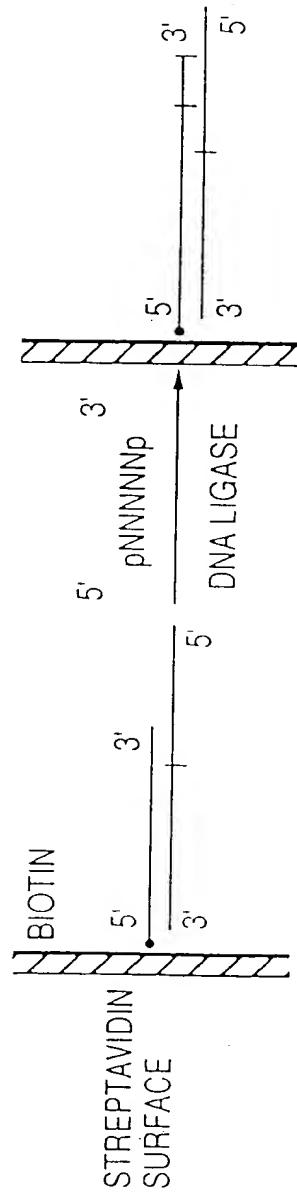
FOUR COLOR ANALYSIS OF SEQUENCE EXTENSION OF THE 3' END OF  
 THE PROBE. LABEL INTENSITY SHOWN DOES NOT INCLUDE CONTRIBUTION  
 FROM THE 3' TERMINATOR.

\* PLUS ADDITIONAL POSSIBLE RESIDUES



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FIG. 10

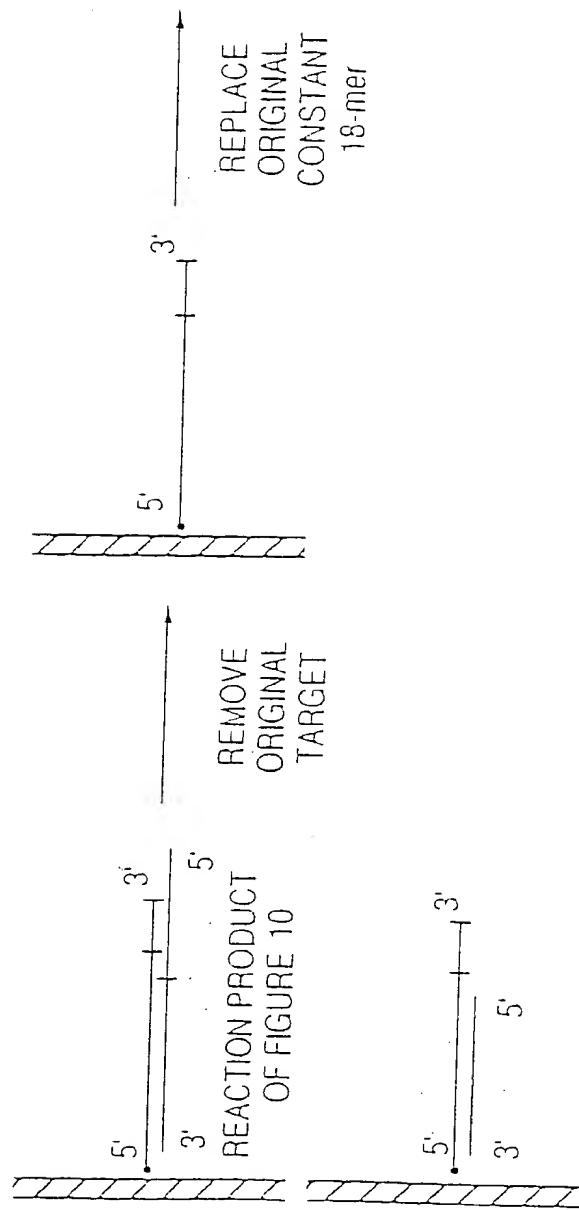


EXTENSION OF THE PROBE BY LIGATION OF A BLOCKED PENTANUCLEOTIDE.  
THE PENTANUCLEOTIDE IS 3'-BLOCKED TO PREVENT POLYMERIZATION.



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FIG. 11



PREPARATION OF A CUSTOMIZED PROBE CONTAINING A 10 bp SEQUENCE  
PRESENT IN THE ORIGINAL TARGET DNA



**FIG. 12**

**PROBE AND TARGET ANNEALED**

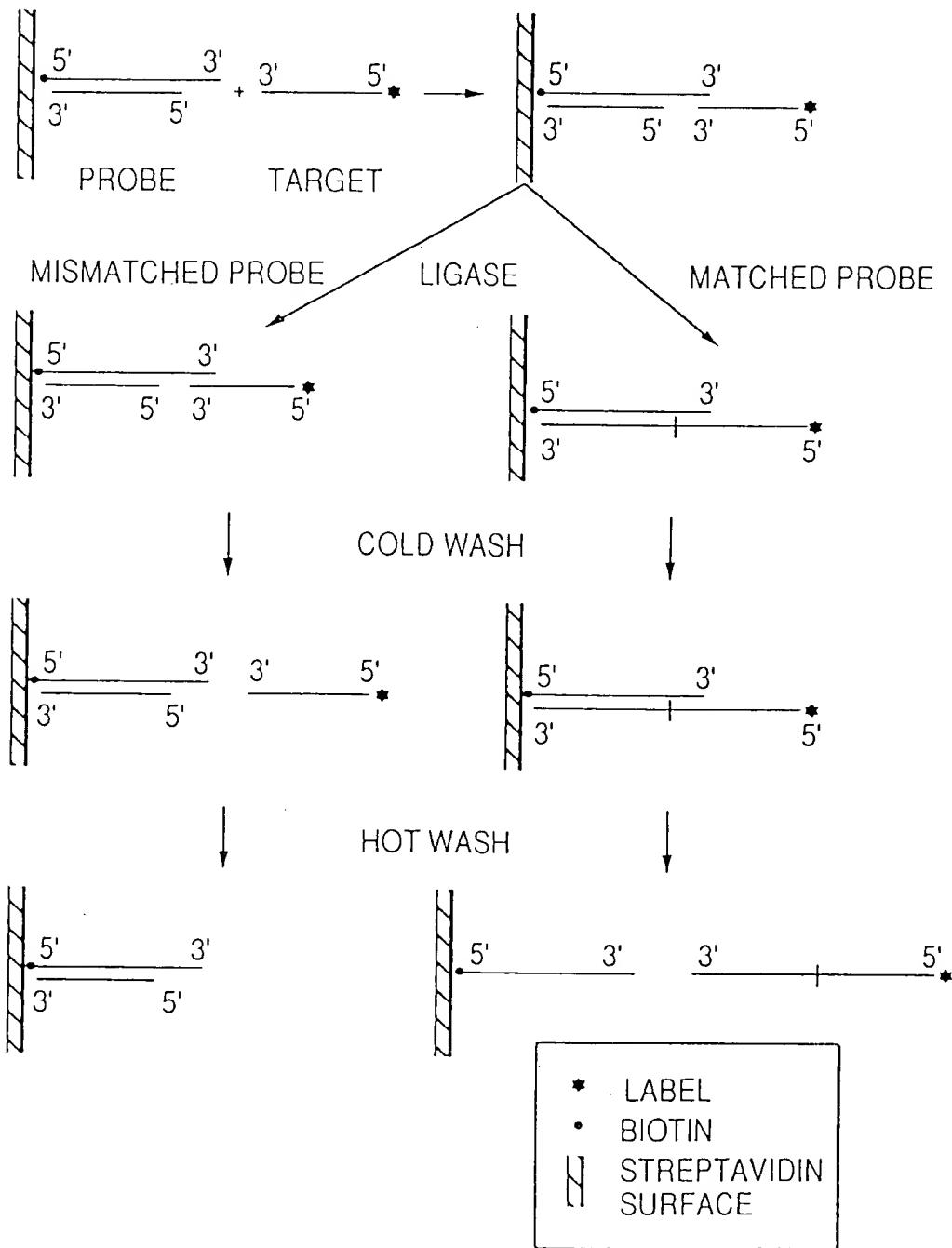
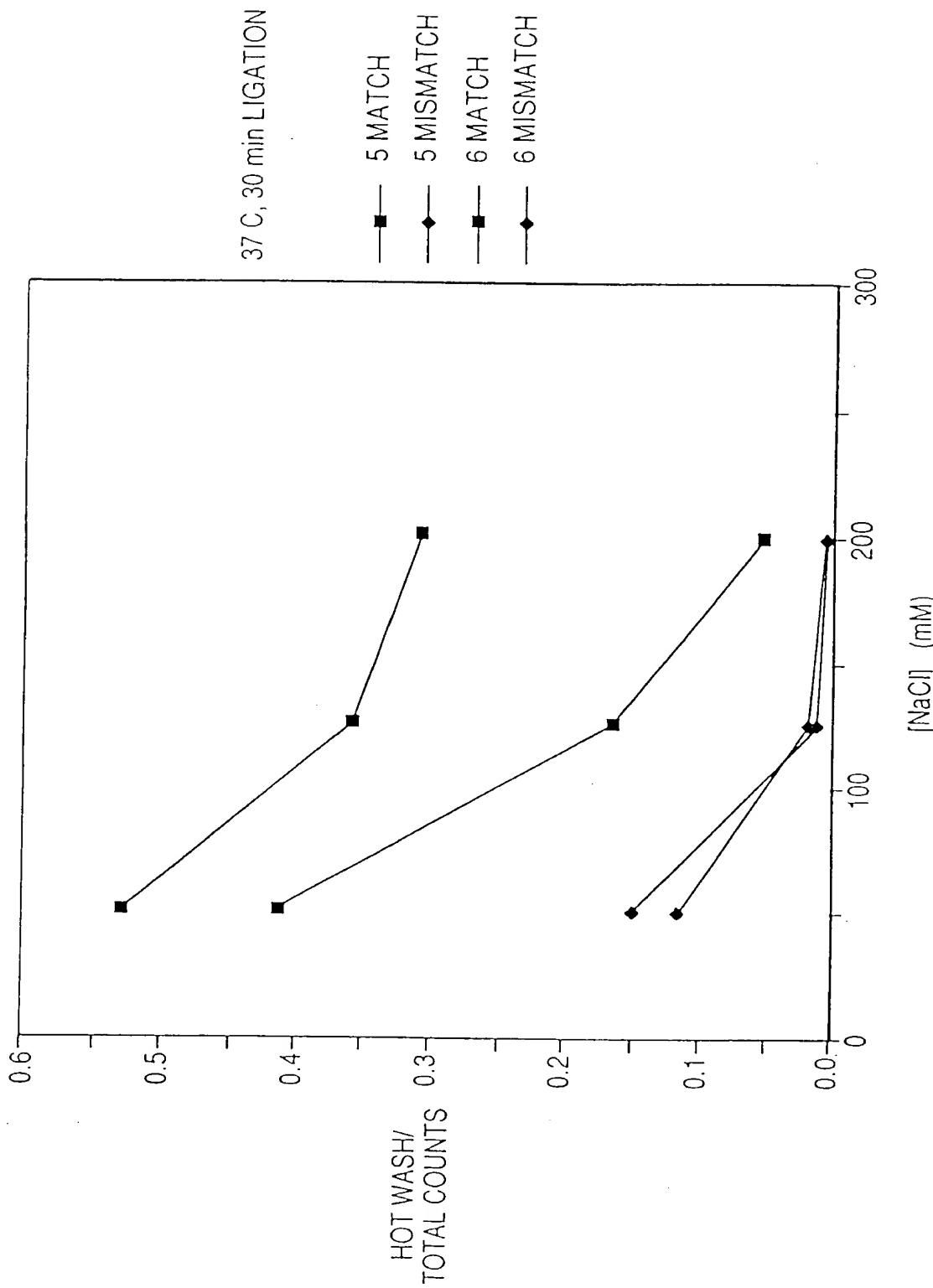




FIG. 13





**FIG. 14**

